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Conformation of protein secreted across bacterial outer membranes: a study of enterotoxin translocation from *Vibrio cholerae*.

Hirst TR, Holmgren J

Department of Genetics, University of Leicester, Great Britain.

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The secretion of enterotoxin by *Vibrio cholerae* is punctuated by the transient entry of the toxin subunits into the periplasm. In this paper, we show that the subunits oligomerize into an assembled holotoxin within the periplasm prior to their secretion across the outer membrane. The rate of toxin assembly was studied by pulse-labeling cells with [35S]-methionine and then monitoring the turnover of radiolabeled subunits as they assembled within the periplasm. The subunits entered the periplasm as monomers and assembled into oligomers with a half-time of approximately 1 min. Since assembly was a rapid event compared to the rate of toxin efflux from the periplasm, which had a half-time of approximately 13 min, we conclude that all of the subunits that pass through the periplasm assemble before they traverse the outer membrane. The average concentration of subunit monomers and assembled holotoxin within the periplasm was calculated to be approximately 20 and approximately 260 micrograms/ml, respectively. This indicates that the periplasm is a suitably concentrated milieu where spontaneous toxin assembly can occur. Our findings suggest that protein movement across bacterial outer membranes, in apparent contrast to export across other biological membranes, involves translocation of polypeptides that have already folded into tertiary and even quaternary conformations.

PMID: 3478701

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Immunoactive chimeric ST-LT enterotoxins of *Escherichia coli* generated by in vitro gene fusion.

Sanchez J, Uhlin BE, Grundstrom T, Holmgren J, Hirst TR

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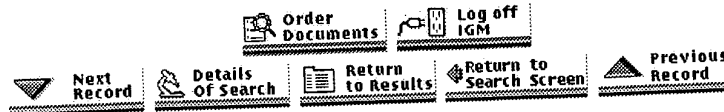
Two different lengths of the gene encoding *Escherichia coli* heat-stable toxin (STa) were fused to the carboxy end of the gene coding for the *E. coli* heat-labile toxin A-subunit (LTA). The hybrid genes directed expression of chimeric LTA-STa proteins. Association of these chimeras with native heat-labile toxin B-subunit (LTB) resulted in protein complexes that bound to GM1 ganglioside and thereby could be assayed in a GM1 ELISA. The complexes reacted with monoclonal antibodies against either LTA, LTB or STa indicating that the STa and LT epitopes remained immunologically intact after fusion. Genetically constructed chimeric proteins exhibiting LT and STa antigens on the same molecule may represent a promising approach to development of broadly protective immunoprophylactic agents and/or useful immunodiagnostic reagents for diarrhoeal diseases caused by enterotoxinogenic *E. coli*.

PMID: 2430831

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TITLE:

Binding specificities of heat-labile enterotoxins isolated from porcine and human enterotoxigenic *Escherichia coli* for different gangliosides.

AUTHORS:

Sugii S; Tsuji T

AUTHOR

AFFILIATION:

Department of Serology and Immunology, School of Medical Technology, Kitasato University, Kanagawa, Japan.

SOURCE:

Can J Microbiol 1989 Jun;35(6):670-3.

CITATION IDS:

PMID: 2670153 UI: 89354010

ABSTRACT:

The binding specificities of heat-labile enterotoxins (LTp and LTh) isolated from porcine and human enterotoxigenic *Escherichia coli* on human erythrocytes were studied by competitive binding assays using different gangliosides as inhibitors. The binding of 125I-labeled LTp to neuraminidase-treated human type A erythrocytes was most effectively inhibited by ganglioside GM1. Ganglioside GM1 was 11 and 105 times more potent than gangliosides GD1b and GM2, respectively. Gangliosides GD1a, GT1b, and GM3 were much less potent. Similar results were also obtained in competitive binding assays with the 125I-labeled B subunit of LTh and neuraminidase-treated human type B erythrocytes, and in those with 3H-labeled ganglioside GM1 and LTp-coupled Sepharose 4B. The binding of 3H-labeled ganglioside GM1 to LTp was not effectively inhibited by galactose-beta(1----3)N-acetyl-D-galactosamine at the highest concentration used. These findings suggest that the combining sites of LTp and LTh may be specific for at least the galactose-N-acetyl-D-galactosamine-galactose (N-acetyl-neuraminic acid) portion of ganglioside GM1.

MAIN MESH
HEADINGS:

Bacterial Toxins/*metabolism
Enterotoxins/*metabolism
Escherichia coli/*metabolism
Gangliosides/*metabolism

ADDITIONAL MESH
HEADINGS:

ABO Blood-Group System
Animal
Binding, Competitive
Comparative Study
Erythrocytes/drug effects

Erythrocytes/metabolism
Escherichia coli/isolation & purification
G(M1) Ganglioside/metabolism
Human
Neuraminidase/pharmacology
Protein Binding
Species Specificity
Support, Non-U.S. Gov't
Swine/microbiology
1989/06
1989/01

PUBLICATION TYPES: Journal Article

CAS REGISTRY 0 (ABO Blood-Group System)
NUMBERS: 0 (Bacterial Toxins)
0 (Enterotoxins)
0 (Gangliosides)
0 (enterotoxin LT)
37758-47-7 (G(M1) Ganglioside)
EC 3.2.1.18 (Neuraminidase)

LANGUAGES: eng



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